ABSTRACT: Pyrite is present in significant abundance in Hanford 300 area (300A) sediments, and understanding oxidation pathways of this mineral can lend insight into the potential for production of ferric iron and sulfate, whose fate may impact contaminant mobility and overall biogeochemical cycling. The ability of microorganisms to catalyze the oxidation of pyrite (FeS$_2$) at circumneutral pH is poorly understood, despite the fact that this an energetically favorable process analogous to various well-known chemolithotrophic pathways (e.g. ferrous iron, sulfide, and elemental sulfur oxidation). Neutral pH abiotic oxidation of pyrite by oxygen has been extensively studied; however, there is virtually nothing known about the capacity for microorganisms to compete with abiotic reactions or enhance rates of pyrite oxidation at circumneutral pH.

This study examined the potential for microorganisms in Hanford 300 Area sediments to oxidize synthetic framboidal pyrite with oxygen or nitrate as the electron acceptor. The work was conducted as part of a larger experiment designed to assess the potential for biotic or abiotic oxidation of natural and synthetic reduced Fe and S phases in Hanford 300 Area sediments. Neutral-pH chemolithoautotrophic enrichment cultures were amended with synthetic framboidal pyrite and inoculated with sediment from at or below the redox transition in 300A sediments. The synthetic pyrite was washed extensively with 6M HCl and acetone to remove potential FeS and S$^0$ contaminants. XRD analysis revealed the presence of pyrite with small quantities of marcasite; no other Fe or S phases were detected. This result is consistent with the observed 1:2 Fe:S ratio of the synthetic framboids. SEM analysis revealed that the µm-sized framboids consisted of aggregates of much smaller (5-10 nm) crystallites.

Several aerobic enrichment cultures capable of chemolithoautotrophic oxidation of synthetic framboidal pyrite linked to sulfate generation were recovered. The extent of sulfate generation in the cultures was several-fold higher than in parallel sterile (uninoculated) controls. No significant sulfate generation was observed in cultures with nitrate as the electron acceptor. Activity of the aerobic cultures was sustained through more than 15 successive transfers over 400 days. The amount of sulfate generated indicated 10-20% oxidation of pyrite. The ratio of Fe(III) to total Fe in 0.5M HCl extracts of the solids declined over time, consistent with concomitant Fe and S oxidation. TEM analysis showed thin coatings of amorphous Fe(III) oxyhydroxides on oxidized framboids, and linear combination fit analyses of Fe K-edge EXAFS showed increased concentrations ferrihydrite and decreased FeS$_2$ concentrations in the oxidized pyrite. No distinct S-bearing mineral phases were detected by SEM, TEM, or XRD. S K-edge XANES confirmed the absence of S in anything other than the -2 oxidation state, consistent with complete oxidation of FeS$_2$ to sulfate.

DNA-based staining and fluorescence microscopy, cryo-SEM, and Fluoresce In-Situ Hybridization (FISH) showed that microbial cells were intimately associated with the pyrite grains. FISH revealed association of both Alpha- and Betaproteobacterial communities with pyrite grains. The composition of the enrichment cultures was assessed using conventional 16S rRNA gene clone libraries. Consistent with the FISH results, the clone libraries were dominated by Alphaproteobacteria (Bradyrhizobiaceae, Mesorhizobium) as well as organisms related to the Betaproteobacterial genus Ralstonia. A culturing
campaign isolated a novel Bosea strain (Bradyrhizobiaceae) capable of chemolithoautotrophic growth through thiosulfate oxidation. Assessment of the ability of the thiosulfate-oxidizing Bosea strain to oxidize pyrite is underway. This research was supported by the SBR SFA at Pacific Northwest National Laboratory.